

REMARKS

Claims 64, 81, 88 and 89 have been cancelled and claims 63, 65, 72, 73, 74, 79, 80, 81, 82, 87 and 90 have been amended. Before responding to individual points that the Examiner has put forward in the office action dated March 10, 2004, applicants submit it would be helpful to the Examiner to clarify two separate issues.

1. Trans-slicing is separate and distinct from intein mediated ligation.

The response to the Examiner on June 27, 2002 stated on page 10, that the Examiner and the Supervisory Examiner agreed that trans-splicing is patentably distinct from the present claimed invention. Applicants are unaware of any new reason why this rejection should be made again.

A cartoon summarizing trans-splicing is attached herewith. It can be seen from the cartoon that trans-splicing requires two specific fragments from a single intein where each of the two fragments is fused to separate proteins and where the two fragments of intein can recombine and thereby activate a splicing reaction. The bringing together and joining of two different proteins is mediated by an intein reaction. Thiol reagents play no part in this reaction.

In contrast, intein mediated protein ligation as claimed utilizes intein cleavage to generate proteins or peptides with a C-terminal thioester and an N-terminal cysteine or selenocysteine. Once cleaved, the intein plays no further role. It is not involved in ligating the two proteins or peptides together. The distinct steps in claim 65 do not suggest trans-splicing in any

way . In particular, steps (d)-(f) of claim 65 require a thiol reagent added to the first fusion protein to form a C-terminal thioester following intein cleavage (d), and cleavage of a second intein from the second fusion protein resulting in an N-terminal cysteine or selenocysteine (e); and (f) permitting ligation of these proteins.

(2) The application is enabled for intein mediated protein ligation using unmodified and modified inteins.

The present claimed invention is directed to a new use of an existing methodology. The existing methodology is intein cleavage using modified (Comb et al) or unmodified inteins (Telenti et al.). The new use is the application of this methodology, in the first place, to forming a protein having an N-terminal cysteine or selenocysteine by intein cleavage, and secondly to ligation, where each component of ligation is derived from an intein cleavage reaction.

It appears from the Examiner's summary on pages 3-5 of the office action dated March 10, 2004, that he recognizes that intein cleavage of unmodified inteins is adequately described in the Telenti reference, Table 1. The Examiner correctly concludes that modified or unmodified inteins can give rise to cleavage in the absence of splicing depending on its context.

Certain statements made by the Examiner in his summary are addressed below.

(i) Page 3, line 13-16, the sentence starting with Telenti et al. refers to splicing. It should be noted that splicing is context dependent as described by Telenti et al.

Evans et al.
S.N.: 09/249,543
Filed: February 12, 1999
Page 12

- (ii) Page 4, line 4-5 is correct for the Telenti reference when referring to MIEP which favors C-terminal cleavage.
- (iii) Page 4, lines 7-9 is also correct when referring to MIEP as starting material.
- (iv) Page 4, line 10-14. Applicants do not understand what the Examiner is describing in this sentence and therefore cannot agree or disagree with its meaning.
- (v) Page 5, line 13 states an exonuclease. This should be an endonuclease.
- (vi) Page 5, lines 15-25. These 10 lines are a single sentence with numerous clauses. Applicants have found it difficult to interpret the literal meaning of this sentence and therefore cannot agree or disagree with its meaning.

Response to Individual Bases of Rejections of Claims

Office Action: Page 2: Objection to claim 90: This claim has been amended. Applicants thank the Examiner for his suggestions.

Office Action; Page 3-5: The Examiner has summarized his understanding of Telenti et al. and agrees with applicants that the present claimed invention is fully enabled.

Office Action: page 6-9: Double patenting

The Examiner has rejected claim 73 under

- (a) provisional obviousness type double patenting over claims 22-25 and 27-30 of co-pending Application No. 09/786,009;
- (b) provisional obviousness type double patenting over claim 96 of US patent 5,834,247.

Claim 73 has been amended to introduce into the method, the step of forming a protein having an N-terminal cysteine or selenocysteine after intein cleavage and prior to ligation with a protein having a C-terminal thioester. There is no suggestion of a method of amended claim 73 in the '247 patent and hence applicants respectfully request that the rejection be reversed.

The Examiner has rejected claims 65 and 69-73 under obviousness - type double patenting as being unpatentable over claims 56, 57, 59 and 60 of US Patent 5,834,247 ('247) on the basis of trans-splicing which the Examiner mistakenly asserts to be indistinguishable from the present claims.

With reference to the claims of the '247 patent: claim 56 requires an element (c), "contacting the first and second modified proteins under a condition suitable for cleavage of the controllable intervening sequence in trans". Claims 57, 59 and 60 are dependent from claim 56 in the '247 patent. The requirement that is underlined above is absent, both actually and inherently, from the claimed invention and is distinct from the requirements of the claimed invention.

Trans-cleavage is not used in the present claimed invention. Instead claim 65 of the present application requires:

....(d) adding a thiol reagent to the extracellular preparation of the first fusion protein whereby the first intein is cleaved so as to form a C-terminal thioester on the first target protein;

(e) cleaving the second intein or modification thereof from the second target protein in the extracellular preparation of the second

fusion protein and forming an N-terminal cysteine or selenocysteine on the second target protein; and

(f) permitting ligation of the first target protein with a c-terminal thioester with the second target protein of step (e).

The claimed method is separate and distinct from claim 56 of the '247 patent.

The obviousness-type double patenting rejection is based on a misunderstanding of the nature of trans-splicing as described in more detail above. '247 patent describes trans-splicing in Examples 12, 13 and 16 and in Figures 25 and 26 but does not suggest or teach the present claimed invention. Unlike the claimed ligation methods, trans-splicing requires that the intein fragments are covalently attached to the proteins to be joined and these intein fragments cause splicing of the proteins. The present claimed methods describe ligation of two proteins in a step that is independent of any intein.

Consequently, applicants respectfully submit that the double patenting obviousness-type rejection is improper and request that it be reversed.

The Examiner has rejected claims 66 and 67 under a judicially created doctrine of obviousness type double patenting as being unpatentable over claims 56, 57, 59 and 60 of US Patent No. 5,834,247 in view of Smith. The rejection is based on a misunderstanding about trans-splicing as described above. The Examiner refers to col 3, lines 25-33 in support of the rejection. However, this refers to an IVPS or CIVPS that is a component in a fusion system used in protein purification. The present claims are not directed to

trans-splicing and the intein is not a component in the ligation of two proteins as claimed herein.

Applicants respectfully request that the rejection be reversed.

Office Action: page 9-18: 35U.S.C.§112

(a) The Examiner has rejected claims 63, 64, 81 and 89 because:

(i) Lack of support for the newly added phrase "other than methionine". This phrase has been removed hence the claim is allowable. The Examiner has also rejected claims 63, 65-72 and 82-89 based on the use of this phrase. This rejection is now moot also.

(ii) Claim 81 stands rejected for same reason as claim 21 previously stated. Cyclic proteins made by methods of claim 65-73 are exemplified and apparently would be considered, but a generic cyclic protein as claimed in claim 81 is objected to. Claim 81 has been canceled without prejudice so that the rejection is moot.

(iii) Claim 89 directed to polymeric proteins are objected to for the same reasons as in (ii). Claim 89 has been canceled without prejudice so that the rejection is moot.

(b) Claims 65-72 and 82-89 are rejected because of the use of the term "plurality of". The Examiner accepts that "plurality" means "more than one of". The Examiner points out that the body of the claim refers to "a first" and "a second". The Examiner states there is no teaching for "a third" or "a fourth" or other number or type of protein or peptide to be ligated and

rejects the claims on the grounds of enablement. Applicants disagree and assert that a skilled artisan reading the present application, would know how to join a third and fourth protein or indeed "n" proteins as appropriate to form a desired polymer. Nonetheless, the term "plurality" has been removed from the claims because it is redundant as it would be clear to a skilled artisan that ligation of a plurality of proteins is inherent in the claimed method. The rejection concerning the use of the term is now moot.

(c) The Examiner has rejected claims 72, 79 and 87 because of the use of the term "cell type". The Examiner proposes that amending claims 72, 79 and 87 to recite "a host cell" will overcome the rejection. Applicants thank the Examiner for his suggested amendment which has been introduced into the present claims so that the rejection should now be reversed.

(d) The Examiner has rejected claims 74, 76-82 and 86-89 because the Examiner asserts lack of enablement with respect to forming cyclic proteins or polymeric proteins. However, in an interview with the Examiner and the Supervisory Examiner, as reported in a response dated August 20, 2003, it was agreed that

the methods described in the Application are sufficient to enable a skilled artisan to form cyclic and/or polymers of a protein by forming a C-terminal thioester on one end of a protein and an N-terminal cysteine or seleno-cysteine on the other end of the protein.
(Page 13 of the response 8/10/03).

No new basis is provided for reversing this conclusion.

Unspliced precursor molecules of protein fused to unmodified or modified inteins at either end of a protein can be obtained as disclosed in the above application and in the Telenti reference. The examples in the application describe the results of cleavage of inteins at either end of a single protein. In particular, this is demonstrated using modified inteins. However, the use of unmodified inteins described in the application for this novel application is fully enabled with respect to the prior art Telenti et al. reference. Accordingly, cleavage of a single unmodified intein at the N-terminal end can be achieved, for example, by using the temperature and incubation times described by Telenti et al. for MIEP. Cleavage of the second unmodified intein at the C-terminal end of the protein can be achieved in the presence of a thiol reagent (DTT) as described by Telenti et al.

Moreover, with respect to enablement, it is well within the knowledge of a person of ordinary skill in the art to appreciate those conditions under which proteins with reactive ends can form a polymer by intermolecular ligation or can form a circularized molecule by intramolecular ligation. An example of a condition that can determine whether circularization or polymerization can occur is concentration of the proteins in a preparation. A dilute preparation would favor circularization while a concentrated preparation would favor polymerization. It is well within the knowledge of a person of ordinary skill in the art to be able to separate circular molecules from polymers by for example, size separation.

The Examiner points out teachings on page 4, lines 7-10, which state that proteins can be isolated with a C-terminal thioester or an N-terminal amino acid residue such as cysteine for use in peptide ligation **or alternatively** both N-terminal amino acid and C-terminal thioester can be created on the same protein for cyclization or polymerization. There is no suggestion here or elsewhere that would suggests the mechanism for forming the desired terminal groups by intein cleavage at one end of a protein should differ for a single protein adjacent to 1 intein at the specified end compared with a protein adjacent to 2 inteins (one at each end of the protein). In each case, N-terminal cleavage and C-terminal cleavage can be achieved as described in the above application.

The Examiner states that Page 10, line 5-page 12 and page 12, lines 19-25 only support cyclization or polymerization with modified inteins. However, the application also describes the use of wild-type inteins for N-terminal or C-terminal cleavage or both (for Example, page 11, line 7 or page 13, line 1). The Examiner agreed with the Applicants that the Telenti reference established the utility of unmodified inteins in cleavage reactions in the absence of splicing. The present claimed invention describes a new use for this established property.

Office Action pages 18-26: Novelty and obviousness

Claim 63 has been amended to incorporate the limitation of claim 64.

(a) Claim 63 is rejected under 35U.S.C.§102(a) as anticipated by Telenti et al. and under 35U.S.C.§102(e) as anticipated by Comb et al. ('247). Applicants assert that claim 63 both prior to and after amendment is separate and distinct from the cited references. However, to facilitate

prosecution, claim 63 has been amended as described above. The cited references describe intein cleavage but do not provide a method for generating a specified amino acid at the N-terminus of a protein as required in claim 63. In fact, the Comb reference teaches obtaining a specified amino acid at the N-terminus of a peptide can be achieved by chemical synthesis or proteolytic cleavage. The Telenti et al. reference does not teach or suggest the desirability of obtaining a protein with a specified amino acid. In particular none of the references suggest or teach the claimed method.

(b) Claim 81 is rejected under 102(e) as anticipated or obvious in view of Albericio. Claim 81 has been canceled without prejudice so that the rejection is moot.

(d) Claim 89 is rejected under 103(a) as unpatentable over Stevens in view of Albericio et al. Claim 89 has been canceled without prejudice so that the rejection is moot.

(e) Claims 64, 65 and 69-73 are rejected under 35 USC 103(a) as being unpatentable over Comb et al. citing col. 16, line 49-col 17, line 51 and col 16, line 59, col 17, line 52-67, col 18, lines 1-41, col 18, line 22, Examples 18-20, col 42, lines 10-47 (claims 63, 65 and 73) in view of Kent et al., Tam or Canne. Claim 64 has been cancelled.

Col 16, line 49 -col 17, line 51 of the Comb et al. reference describes trans-splicing. For the reasons provided above, trans-splicing is separate and distinct from intein mediated protein ligation. The combination of the description of trans-splicing with references by Kent, Tam or Canne does not

teach the present claimed invention. Col 18, lines 1-41 are directed to splicing, more particularly trans-splicing and not to cleavage.

Col 42, lines 10-47 of the Comb et al. reference describe production of an MIP fusion protein in which splicing was completely blocked with cleavage at one splice junction only. This system was designed so as to purify target proteins by means of an associated binding protein (MBP) on affinity columns and then cleave the target protein from the affinity binding protein by intein cleavage. This does not teach or suggest a method of ligation described in claim 65.

Examples 18-20 of the Comb patent are directed to forming a C-terminal thioester on a protein as a result of intein cleavage in the presence of specified thiol reagents. Purification of proteins from an affinity column is also described in these examples. There is no suggestion or teaching of ligating proteins in these examples as required in claim 65 and dependent claims 69-72 or in claim 73 in the present invention. Nor is there any suggestion of forming a protein with an N-terminal cysteine or selenocysteine by intein mediated cleavage as required by claim 63 of the present invention.

The combination of Canne, Kent or Tam with Comb et al. even if there was a suggestion or motivation to combine, would not describe the present claimed methods because none of these references suggest or teach a method of forming a protein having an N-terminal cysteine or selenocysteine by intein mediated cleavage of a fusion protein. Nor does any such combination describe ligation of proteins which bear a C-terminal thioester

Evans et al.
S.N.: 09/249,543
Filed: February 12, 1999
Page 21

or an N-terminal cysteine derived from cleavage of target proteins fused to inteins and expressed in a host cell.

Consequently, Applicants respectfully request that the rejection be reversed.

Evans et al.
S.N.: 09/249,543
Filed: February 12, 1999
Page 22

CONCLUSION

For the reasons set forth above, Applicants respectfully submit that the rejections set forth in the Official Action of March 10, 2004 have been overcome and that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension of time in which to file this response. Enclosed is check in the amount of \$640, covering the \$475 extension fee and the fee of \$165 for filing a notice of appeal.

Applicants authorize that any additional fees that may be due be charged to Deposit Account No. 14-0740.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned collect at the number shown below.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.


Harriet M. Strimpel, D.Phil.
(Reg. No. 37,008)
Attorney for Applicant
32 Tozer Road
Beverly, Massachusetts 01915
(978) 927-5054; Ext. 373

Date: August 17, 2004